Fazekas

September 19 57

Dear Stephen:

I imagine you must have anticipated that I would very rapidly come to answer my own questions of my last letter. I hasten to write again know to minimise your bemusement.

As shouldhave been the first order of business, I have been settling down to someintensive random reading— almost every paper has some bearing, intentional or otherwise, on the problem of incomplete virus, which is itself perhaps a sign of the significance of this material for virus maturation.

As I should have learned sooner, the experiment on RDE-treatment, post-infection has been done, e.g. by Henle & Co. ((should there be a designation in scientific business for Pty. Ltd., the latter having just the same meaning as in commerce, limited responsibility?)), in which the yields had much the same infectivity ratio regardless of the treatment. By Henle's own account, the numbers of eggs were 'adequate', andin any case, we have had the same result in a crude trial, so it isprobably correct. I would still see some point, however, in a more precise trial in which the interaction of input dose with RDE treatment, etc., could be better defined.

However, this result, while giving no support to The Hypothesis, is not fatal to it, and I am rather impressed with two recently assimilated facts: 1) that RDE removes little of the membrane-bound HAi(nhibitor), and 2) that heated virus still adsorbs to RDE-treated membranes (Karr & Schlesinger: may I rely on this?) Cairns suggests that most of the bound HAi is on or beyond the mesodermal surface of the allantoic cells, which gives a convenient refuge of untestability for certain hypotheses. (Have you by the way looked at the effect of periodate on the bound HAi?)

Even more interesting are the studies (Hoyle, Henle Co. etc) on the incidence of HA, virus etc. in the infected membranes, and I am rather impressed with the implication that the developing virus is uninfective until it emerges -- again the notion that its own skin comprises the cell surface. In fact, Hoyle has stated a view of incomplete virus which is quite close to The Hypothesis (cf. J Hyg 48, p. 296).

(I'm a bit confused whether you and Cairns agree that the membrane virus has a markedly lower infectivity ratio than that liberated into the medium--cf Henle No. 14, JEM 103:799)

Of course this is just one of several plausible versions of the critical maturation step that may be defective in the formation of IV.

I have not seen many experiments more appalling than figure 2 of Henle No. 10, but it does seem to me rather odd that Cairns' proposition of IV yield after low multiplicity should not have been paralleled in other studies, even allowing for imperfect measurements of the amount of virus actually adsorbed. I thought at first this discrepancy might have something to do with washing out the egg, but similar results are reported for empty eggs.

This leads me to ask whether you can give me a more detailed account of your experiment on effect of allantoic fluid. (We have been finding that undiluted allantoic fluid is quite inhibitory to the growth of PR8 in empty eggs, as is egg white at 1:10. Ditto for LEE. (We are setting this up as a selective method for the C marker, with preliminary results quite encouraging.) I have been putting as much as 20 Ads (i.e. in this lab's terminology, viz. 5 ml of fluid diluted to HA=20) into empty eggs and getting out quite infective yields. This is in Ringer's.) I have been trying rather hard to uphold the approach to IV that you were leading me to in our discussion, and I find I am relying on my rather casual recollection of this particular experiment. If it could be repeated here it would convince some of the die-hards.

As to periodate, I am much worried by Henle's contention that 'glycerol-neutralized' periodate will still inactivate flu. Is there a flaw here? Is that inactivation the irreversible combination of modified allantoic receptor with the virus? (This is Henle No. 13)

Finally, on the theoretical side, if its is true that a later (+ 2 hours) superinfection with inactivated virus can provoke the yield of IV, this would tend to argue against an event at the time of initial entry as being critical. This experiment (again Henle 13) reads to me rather better than some of the others, but I was not well impressed with some features of table III, e.g., the timing and the expectation that NIHA produced after 12hours could be assayed in the presence of the earlier accumulation. Your remarks on the reliability of the result, and if true, its interpretation would be of great interest.

I can foresee that we are going to have to make a stab at the bit technique, and if not here certainly at home, so I would like to take you up on your offer to send some of the 'gear'. I'm sure you know what's on hand here, e.g., if nothing else some reliable gelatin.

When I ask for as much as I do in this letter, I should enclose a 'requisition', and so I do.

Yours,

Joshua Lederberg